NORTHRUP EXHIBIT G

Please file...

B92-011

Mr. Scott Taper
Office of Technology Licensing
University of California
2150 Shattuck Ave. Suite 510
Berkeley, CA 94720

Dear Scott,

Enclosed is a continuation of Professor White's and my disclosure entitled "Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics".

As I mentioned on the phone, I feel that it is important to extend the application of the first disclosure to include pre-and post-PCR reactions and manipulations with microdevices. I hope the enclosed is understandable to you. If not, I will be happy to go over it with you in greater detail.

Sincerely,

M. Allen Northrup

R. M. White

Richard M. White

Disclosure Continuation

Re: Microinstrumentation-Based Polymerase Chain Reaction (PCR) Diagnostics

Date:_____

Inventors:

M. Allen Northrup_____
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Subject:

PCR in a microdevice (i.e., micro-fabricated through process technology), can be one step in a series of manipulations and conditions leading to the diagnostic detection of a variety of target species and the use of PCR products in genetic engineering. Amplification via PCR, as manipulated and controlled with micro-systems, yields products themselves that are subject to enhancement and detection with such devices. The control of reactions and conditions, and the physical manipulation of pre-PCR, PCR, and post-PCR products and reagents are the subject of this continuation.

The physical and chemical control via microdevices of cells and reagents prior to and after the production of PCR products will enhance the potential applications of DNA-based processes and analyses. Pre-PCR manipulation of target cells or microorganisms can also be accomplished with microdevices. One example of many possible treatments may include physical and/or chemical inducement to cell lysis. The use of the properties of the device (e.g. ultrasonic waves, surface states, and coating materials) can be used to manipulate cells and cell-contents, as can chemical treatments created by stirring and or mixing reagents from other areas and volumes on the integrated microinstrument. Sonication in conjunction with microparticles, for example, has been used to expedite the extraction of DNA from fixed cells on macro-scale

(Heller et al). Strategies similar to this, but relying on the inherent properties of a microdevice can be used to prepare intact cells, microorganisms, tissues, and other analytical samples for PCR and subsequent techniques. Two examples of many possibilities of this are the use of ultrasonic waves to disrupt and expose cell components through lysis, and to unravel large or long chain molecules such as DNA and proteins via disruption of secondary structure. Physical and chemical treatments such as these can also be incorporated into the PCR and post-PCR phases of microdevice-based treatments to augment the reactions *in situ*.

The use of microdevices to perform PCR is the subject of the previous disclosure by the present inventors (May 2, 1991). However, it is the intention of the present disclosure to extend the previous disclosure to pre- and post-PCR treatments. Potential post-PCR treatments are numerous, but the principle with a few examples will be described herein. In general, PCR on a microdevice is an integral part of the potential application of a device to further biotechnological manipulations and analyses. In other words, once PCR has been performed on a microdevice, post-PCR manipulations can lead to a myriad of possible microdevice-based DNA analyses and treatments. A few examples of such analyses are: large- and small-scale DNA sequencing of target species, cell-typing, analysis of PCR products with DNA probes, DNA fingerprinting, DNA cloning, physical mapping of genes and the maintainence of DNA libraries. Such analyses can lead to the use of DNA as vectors to produce cells or other biological entities that can produce desired products such as proteins or other therapies, or it can be used to produce DNA for use in therapies or biotechnological processes.

Exposure of PCR products to sequences of target DNA, or synthetic analogues in microdevices can be accomplished with the manipulative capabilities of microfabricated electrical and mechanical machines. For example, multi-dimentional fields of pre-determined DNA sequences (probes) can be exposed to PCR products (e.g., by physical pumping or electrophoretic methods) and their subsequent analyses can be accomplished with microdevices. Immobilization of biochemical molecules onto electronic devices and subsequent detection has been accomplished (Pharmacia, Molecular Devices, NRL). The integration of similar surfaces within micro-device based integrated systems is one example of the present continuation. Direct DNA sequencing of PCR products (single or double-stranded), can be accomplished with the use of unique

temperature, enzymatic, strand separation schemes, and detection methodologies; all of which can be incorporated into a microdevice. Detection windows, reflective and absorptive surfaces, optic sources and other optical components can be fabricated and integrated onto a micro-device instrument, providing optical detection methodology. Signal output and data analyses can be accomplished with on-board electronics.

PCR products may also be manipulated in order to be incorporated into genetic engineering vectors such as plasmids. Such vectors may subsequently be incorporated into target cells for the production of desired compounds. The target cells or moieties and reagents can be stored in reservoirs on the integrated device, released for exposure to the vectors while the physical/chemical conditions are established with the device. One other potential application would be the *in situ (in vitro or in vivo)* release of PCR products for direct genetic therapy or manipulations.

In summary, this continuation describes the extension of microinstrumentation-based polymerase chain reaction (PCR) diagnostics to include pre- and post-PCR-product manipulations to release target DNA, and continue the treatment of PCR products for a large potential variety of genetic engineering tasks. These tasks include examples such as but not limited to, incorporation of genetic vectors, DNA recombination, cell cloning, genetic therapy, and treatment and testing of biotechnological processes. As well, the incorporation of detection methodology, such as but not limited to, optical methodology, is included as it pertains to the the detection of pre-, concurrent, and post-PCR-processes on an integrated PCR-based microinstrument. The use of micro-manipulation and treatment of PCR-based technologies in microdevices can allow highly automated DNA technologies due to its ability to perform many reactions and manipulations with precise control of temperature, evaporation, small-volume reagent delivery, product separation and isolation, and *in situ* reaction and product detection in parallel. Such highly automated and in parallel technologies will go far to expedite the use of DNA-based technologies for biomedical (e.g., the Human Genome Project), environmental (e.g., contaminant identification) and industrial (e.g., biotechnology) applications.